AD_____

Award Number: DAMD17-01-1-0069

TITLE: The Clinical Development of Thalidomide as an Angiogenesis Inhibitor Therapy for Prostate Cancer

PRINCIPAL INVESTIGATOR: Christopher J. Logothetis, M. D.

CONTRACTING ORGANIZATION: The University of Texas
M.D. Anderson Cancer Center
Houston, TX 77030

REPORT DATE: October 2004

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

# REPORT DOCUMENTATION PAGE

*Form Approved*
*OMB No. 0704-0188*



| 1. REPORT DATE | 2. REPORT TYPE | 3. DATES COVERED |
|---|---|---|
| 01-10-2004 | Annual | 28 Sep 2003 – 27 Sep 2004 |

**4. TITLE AND SUBTITLE**

The Clinical Development of Thalidomide as an Angiogenesis Inhibitor Therapy for Prostate Cancer

**5a. CONTRACT NUMBER**

**5b. GRANT NUMBER**
DAMD17-01-1-0069

**5c. PROGRAM ELEMENT NUMBER**

**6. AUTHOR(S)**

Christopher J. Logothetis, M. D.

**5d. PROJECT NUMBER**

**5e. TASK NUMBER**

**5f. WORK UNIT NUMBER**

**7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)**

The University of Texas
M.D. Anderson Cancer Center
Houston, TX 77030

**8. PERFORMING ORGANIZATION REPORT NUMBER**

**9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)**
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

**10. SPONSOR/MONITOR'S ACRONYM(S)**

**11. SPONSOR/MONITOR'S REPORT NUMBER(S)**

**12. DISTRIBUTION / AVAILABILITY STATEMENT**
Approved for Public Release; Distribution Unlimited

**13. SUPPLEMENTARY NOTES**
Original contains colored plates: ALL DTIC reproductions will be in black and white.

**14. ABSTRACT** The purpose of this award is to evaluate the: 1) Safety and toxicity and neo-adjuvant thalidomide therapy prior to radical prostatectomy in patients with locally advanced prostate carcinoma (PCa); 2) Efficacy of neoadjuvant thalidomide treatment, as measured by the rate of tumor reduction/PSA decline; 3) Qualitative measurements of the in vivo effect of thalidomide therapy on the Endothelial and Epithelial compartment. Significance: The ability to assess in vivo the effects of thalidomide as well as identify surrogate markers of anti-angiogenic activity is invaluable to the design of new effective therapies. Prior to the DOD's final decision (April 2002) to sponsor the correlative studies proposed in the grant 18 patients had been entered in this study. Their clinical information I attached. Pending some additional HSRRB required amendments, the patients' research tissue was stored but the funds awarded were not used to perform any of the studies until we obtained the final DOD approval. After April 2002 (when DOD was cited as a partial sponsor) and until we solved all the remaining issues and obtained DOD clearance to proceed (9/2003) we held accrual in this trial. We have re-activated the study and are confident that we will complete patient accrual within 1 year. We are requesting a no-cost extension of 1 year to allow completion of the planned studies.

**15. SUBJECT TERMS**
Angiogenesis inhibition, thalidomide, prostate cancer

| 16. SECURITY CLASSIFICATION OF: | | | | 18. NUMBER OF PAGES | 19a. NAME OF RESPONSIBLE PERSON |
|---|---|---|---|---|---|
| | | | | | USAMRMC |
| a. REPORT | b. ABSTRACT | c. THIS PAGE | | | 19b. TELEPHONE NUMBER *(include area code)* |
| U | U | U | UU | 11 | |

## Table of Contents

## INTRODUCTION

Advances in the technological ability to interrogate treated prostate cancer specimens have been made in 2004 at M. D. Anderson Cancer Center. Parallel to this there have been further advances in the understanding of the pathways implicated in prostate cancer progression and particularly those implicated in the host-epithelial responses. Studies that will expand the scope of pathways implicated in the host-epithelial interaction that may contribute to angiogenesis and may be the target of thalidomide have been developed and approved for study in thalidomide-treated specimens. This expanded study of 18 study specimens will likely inform us as to the effect of thalidomide on the host-epithelial interaction implicated in prostate cancer progression. These expanded studies will be integrated with the initially proposed studies for the interrogation of tumor-associated vascularity as initially proposed. It is anticipated that the original and expanded goals of the study will be completed within the first six months of 2005.

## BODY

### Aim 1. Assessment of safety and toxicity of preoperative thalidomide treatment.

### Methods

Subjects in this prospective study of preoperative thalidomide were men with histologically confirmed prostatic adenocarcinoma with no evidence of regional or distant metastases; disease could be clinical stage T1c-T2c with Gleason score of 7 or higher on initial biopsy or clinical stage T3. All gave informed consent to participate in this phase II study, which was approved by the institutional review board of The University of Texas M. D. Anderson Cancer Center.

Thalidomide was given once daily in the evening at a starting dose of 200 mg/day. This dose was escalated by 200 mg/day every week to a maximum of 600 mg/day if no toxicity greater than grade 2 ensued. Each treatment cycle lasted 42 days (6 weeks). At 6 weeks and 12 weeks, patients underwent digital rectal examination, transrectal sonography, and serum prostate-specific antigen (PSA) testing. If lesions showed no evidence of growth and serum PSA level had not increased at the 6-week interval, thalidomide treatment was continued for a maximum of 3 months (i.e., 2 cycles). PSA progression was defined as an increase in serum PSA of more than 25% over the baseline (pretreatment) value. Progression of measurable intraprostatic lesions was defined as an increase of more than 25% in two dimensions. Radical prostatectomy was performed after termination of the thalidomide treatment. For statistical analyses, the design of Thall, Simon, and Estey was used, and a success probability of 0·20 or larger was considered clinically promising. Clinical success was defined as stable disease (no increase in tumor mass) at 6 weeks followed by a decline in serum PSA of > 50% at 12 weeks. The maximum number of patients to be treated was set at n=40.

### Results

**Total number of patients treated: 18**
Patient's characteristics are outlined in table 1
Total numbers of patients completed and going to prostatectomy are: 15
Total number of patients in whom tissue (prostate) has been collected from prostatectomy and serum are: 15 (15 primaries) (lymph nodes: 2).
Pathologic characteristics of radical prostatectomy specimens are outlined in table 2
Plasma samples have been collected from: 16 patients

*Results:*
Thalidomide was well tolerated and no delay in surgery or preoperative complications occurred.

**Drug related toxicity**: All patients were evaluable for toxicity. Drug dose was escalated to 600 mg thalidomide daily in all patients. Seventeen of the 18 patients (95%) completed treatment as scheduled. The incidence of adverse events ranged from 5% to 61 %. (Table 3). Median time to surgery from thalidomide termination was 5 days (range 2 to 18 days). Prostatectomies were uneventful except for three cases involving difficulties in apical dissection, dissection from the rectum, or both.

### Aim 2. Assess the efficacy of preoperative treatment with thalidomide in patients with locally advanced prostate cancer.

**Methods**

Pre Operative:
1. PSA time course: completed
2. Circulating Factors: pending

**Results**

1. PSA time course (these results have been reported in the previous annual report)
At 6 weeks of treatment, PSA levels were a median 38% lower than at baseline (range, –12% to 49%), with eight patients showing a reduction of at least 40%. At 12 weeks, the median PSA reduction was 42% (range, –19% to 70·9%), and six patients (33%, 95% confidence interval, 16% to 56%) achieved a PSA reduction of at least 50%. Testosterone concentrations remained unaffected. Median testosterone levels were 308·85 ng/dL (range, 186·71–595) at baseline and 341·29 ng/dL (range, 208·88–923·97) at the end of the 12-week treatment period. (Figure 1)

**Aim 3. Obtain qualitative measurements of in vivo effect of treatment**

**Methods**

**Interrogating human tissue has improved dramatically since the submission of the grant in 2001**. These technological advances have been applied to the study of this tissue. These advances permit the expansion of the scope interrogation. We have completed construction of a tissue microarray (TMA) from available radical prostatectomy tissue samples. Areas have been selected from all the available primary tumor foci and adjacent stroma as well as from non-malignant areas-both glandular and stromal- of the peripheral and/or transitional zone where applicable. Additionally, the TMA has been designed to include cores representing the whole spectrum of histologic patterns found on the different tumor foci. With this design we will attempt to further the thorough study of the effect of preoperative thalidomide not only on the tumor as a whole. We can break down our analysis and look for differential effect according to histologic pattern, study the effect on the crosstalk between tumor and adjacent stroma including the vascular compartment, and possibly address the effect on the non-tumor epithelial and stromal compartment.

A second TMA consisting of untreated cases matched for Gleason grade and stage will serve as a control for the evaluation of this effect. (This control TMA will serve to not only compare differential expression or localization of expression of the various factors of interest, but most importantly it will guide the assessment of factors or populations whose expression may be lost after treatment and may not otherwise be identified, particularly since we aim to interrogate pathways and interactions that are currently still under investigation in the prostate cancer context).

In addition to the technological advances we have developed a new insight into the tumor microenvironment at a molecular and cellular level. Thus, we have completed the establishment and accumulation of the data for full characterization of the epithelial- stromal and vascular compartment of the tumor with tissue micro arrays. The epithelial-stromal crosstalk is currently regarded as probably the determining factor of tumor invasion and metastasis. We will study the thalidomide effect in this light. We intend to study the effect of thalidomide in the tumor microenvironment with regard to the endothelial compartment by assessing microvessel density and expression of vascular markers in both the epithelial and stromal compartment. Furthermore we will attempt to determine in a correlative

manner whether broader epithelial- stromal crosstalk is interrupted by treatment by assessing expression of relevant markers such as matrix metalloproteinases, E-cadherin, Transforming growth factor-beta and components of the hedgehog signaling pathway recently implicated in prostate cancer progression Following immunochistochemical staining of the TMA slides. We will use the BLISS system to scan and store digitally the images of the TMA slides stained for the aforementioned markers as webslides in the webslide server. Through the webslide server and by using a highly optimized browser (web slide browser) we access, characterize and score the saved images. This information is stored in an embedded database to be used for statistical analysis.

**Results**

1. We have completed construction of the TMA of thalidomide pretreated patients. The thalidomide pretreated TMA consists of a total of 475 (353+122) cores on two slides. Fifteen cases are represented and 21 different tumor foci. The H&E has been stored digitally and reviewed. The following are the characteristics of the TMA with regards to histologic patterns represented.

   **Thalidomide treated TMA**
   Non-malignant areas
   Peripheral zone represented in 12 cases (67 cores)
   Transition zone represented in 13 cases (73 cores)
   Malignant histologic patterns:
   Single cell clusters represented in 9 cases, 9 tumor foci (54 cores)
   Isolated glands represented in 12 cases, 15 tumor foci (88 cores)
   Fused glands represented in 15 cases, 18 tumor foci (105 cores)
   Cribriform represented in 7 cases, 7 tumor foci (42 cores)
   Papillary represented in 2 cases, 2 tumor foci (12 cores)
   Intraductal represented in 6 cases and tumor foci (22 cores)
   Finally, lymph node metastasis specimens where available in two cases (12 cores)
   We have thus adequately represented the heterogeneity of the tumor

2. We have successfully established protocols for antibody titration for a panel of 27 different candidate markers.

**Key Research Accomplishments**

- Preoperative Thalidomide administration in patients with locally advanced prostate cancer was well tolerated. No perioperative complications or delay in time to surgery occurred.
- Preoperative Thalidomide administration resulted in a PSA response rate of 33%. Median reduction of PSA levels was 42% after 12 weeks of administration.
- We have used the current tissue microarray technology to adequately represent the heterogeneity of prostate cancer as well as the tumor microenvironment. We will employ this accomplishment to interrogate the biologic effects of thalidomide.

**Reportable Outcomes**

Pending

## CONCLUSION

Substantial progress has been made toward completion of the goals of the project. We have assessed the safety and feasibility of preoperative thalidomide in locally advanced prostate cancer. Our data with regard to PSA response are also suggestive of clinical efficacy. These findings have become more important and likely more informative based on three pieces of information regarding its relevance in prostate cancer that will influence its significance. These include; 1. Thalidomide prolongs progression free survival of prostate cancer patients based on the results of a randomized trial 2. Characterization of the epithelial compartment of prostate cancer in human specimens becomes more feasible. 3. New knowledge on the mechanistic basis of the stromal –epithelial interaction that characterizes prostate cancer progression will be incorporated into the study.

## REFERENCES

1. Thall PF, Simon R and Estey EH: Bayesian sequential monitoring dDesigns for single –arm clinical trials with multiple outcomes. Statistics in Medicine 14, 357-379, 1995.
2. Coombes KR, Zhang L, Bueso-Ramos C, Brisbay S, Logothetis C, Roth J, Keating MJ, McDonnell TJ. TAD: A web interface and database for tissue microarrays. Applied Bioinformatics 1: 155-158, 2002.
3. Assikis VJ, Do-KA, Wen S., Wang X, Cho-Vega JH, Brisbay S, Lopez R, Logothetis CJ, Troncoso P, Papandreou CN, McDonnell TJ. Clinical and biomarker correlates of androgen-independent, locally aggressive prostate cancer with limited metastatic potential. Clin Cancer Res 10: 6770-6778, 2004.
4. Jonasch E, Do KA, Logothetis C, McDonnell TJ. Issues and applications for tissue arrays in target and drug discovery. In: Molecular Cancer Therapeutics: Strategies for Drug Discovery and Development. George C. Prendergast, ed. John Wiley and Sons Inc, Hoboken NJ, pp73-90, 2004.
5. Tibbs RF, Abrahams NA, Wang X, Vakar-Lopez F, Pettaway CA, Pisters LL, Mathew P, McDonnell TJ, Do K-A, Troncoso P. Pathological Characterization of Residual Prostate Cancer in the Neoadjuvant Surgical Model: A Proposal for Standardization of Morphologic Features of Treated Prostate Cancer, Modern Path 18 (Supplement 1), 166A, 2005.

**Table 1**. Patient characteristics

| **Patient Characteristics** | |
|---|---|
| **Age (years)** | |
| Median | 60 |
| Range | 43-71 |
| **Clinical Stage T** | |
| $T1_c$ | 2 |
| T2 | 6 |
| T3 | 10 |
| **Gleason score at biopsy** | |
| 7 | 8 |
| 8 | 5 |
| 9 | 4 |
| 10 | 1 |
| **Pretherapy serum PSA levels** | |
| <20 ng/ml | 11 |
| >20 ng/ml | 7 |

**Table 2**. Pathologic Characteristics of radical prostatectomy specimens from thalidomide-treated patients.

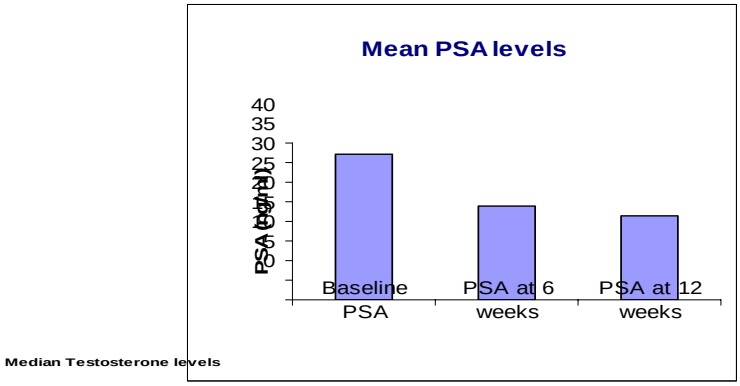
| **Pathologic Stage** | **No. of Patients** |
|---|---|
| **Organ-confined** | |
| **pT2** | 2/15 |
| **Extraprostatic Extension** | |
| **pT3a** | 1/15 |
| **Seminal vesicle invasion** | |
| **pT3b** | 12/15 |
| **Positive lymph nodes** | |
| **pT any, N1** | 4/15 |
| **Positive surgical margin** | 9/15 |
| **Gleason Score** | |
| 7 | 2/15 |
| 8 | 3/15 |
| 9 | 10/15 |

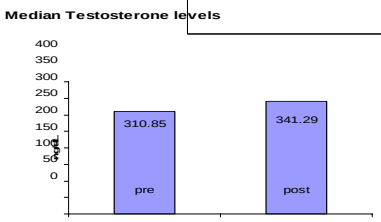
**Table 3**. Toxicities

| **Toxicities** | ***G1*** | ***G2*** | ***G3*** |
|---|---|---|---|
| Somnolence | 10 | 2 | |
| Constipation | 6 | 5 | |
| Fatigue | 6 | 3 | 1 |
| Pruritus | 7 | 2 | |
| Motor/ ataxia/ tremors | 5 | 1 | 1 |
| Xerostoma | 7 | | |
| Dizziness | 6 | 1 | |
| Edema | 6 | | |
| Pain | 5 | 1 | |
| Sensory | 5 | | |
| Blurred vision | 5 | | |
| Diarrhea | 4 | | |
| Vasovagal episode/ bradycardia | 1 | 1 | 1 |
| Cardiac | 4 | | |
| Memory loss | 3 | | |
| Urinary frequency | 3 | | |
| Dyspnoea/ diaphoresis | 2 | 1 | |
| Nausea/ vomiting | 2 | | |
| Taste alteration | 2 | | |
| Confusion | | 1 | |
| Memory loss | 2 | | |
| Insomnia | 2 | | |
| Allergic rhinitis | 2 | | |
| Hypomagnesemia | 1 | 1 | |
| Flatulence | | 1 | |
| Confusion | | 1 | |
| Depression | 1 | | |
| Headache | 1 | | |
| Hypotension | 1 | | |
| Sexual function | 1 | | |
| Dry eyes | 1 | | |
| Hot flashes | 1 | | |
| ***Total*** | ***101*** | ***21*** | ***3*** |

**Figure 1**

# Change in PSA Levels




**PSA drop > 50% = 33%**
**Median PSA reduction at 6 weeks 38.28%**
**Median PSA reduction at 12weeks 41.82%**
**No effect on testosterone**